Prevalence and virulence gene profiles of *Proteus mirabilis* isolated from animal, human and water samples in Krishna District, Andhra Pradesh, India

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Abstract

In the present study, a total of 507 samples comprising food of animal origin (215), faecal swab samples (188), human urine samples (65), human diarrhoeic stool samples (12) as well as water samples (27) were examined. Overall prevalence of *P. mirabilis* was found to be 34.51% (175/507) by species-specific PCR. Among foods of animal origin, the highest rate of *P. mirabilis* isolates were recovered from chicken samples (38.7%), followed by pork (37.5%) and mutton samples (28.9%). Among faecal swabs, the highest rate of *P. mirabilis* isolates were from poultry (49%), followed by pigs (37.8%). Human urine samples showed a prevalence rate of 10.7%. Water samples showed 7.4% prevalence. All the human diarrhoeic stool samples were negative for *P. mirabilis*. Some of the virulence factors genes were investigated using polymerase chain reaction technique. The genes *ureC* (80.5%), *ureA* (72.5%), *flaA* (28.5%), *hmpA* (60.5%) and *zapA* (50.28%) were detected in *P. mirabilis* isolates.

Keywords: *Proteus mirabilis*, prevalence, virulence genes, polymerase chain reaction

1. Introduction

The *Enterobacteriaceae* group of bacteria is the most challenging contaminant to meat and meat products worldwide. *E. coli*, *Salmonella*, *Proteus* and *Klebsiella* species are the most predominant species in all food poisoning cases associated with meat products. *Proteus* spp. are Gram negative rods measuring 1-3µm in length and 0.4-0.8µm in diameter, motile by peritrichous flagella, facultative anaerobic, non spore-forming and non-capsulated with most isolates having fimbriae. *Proteus* species are frequently found in soil, water and the intestinal tract of many animals, including humans (Drzewiecka, 2016 and Nahar et al., 2014) [8, 13].

Many outbreaks of food poisoning have been attributed to organisms of the *Proteus* group and due to the rising incidence of food borne infections caused by *P. mirabilis*, there is an urgent need for control and/or prophylaxis for food poisoning outbreaks associated with meat products. It depends greatly on investigating such agents in foods eliminating them to ensure food safety and to protect public health from microbial contamination of food.

A few cases of food poisoning caused by *P. mirabilis* have been reported in the last several decades (Cooper et al., 2005 and Zietze, 1984) [6, 20]. They have been isolated from chicken, beef, pork and poultry droppings (Nahar et al., 2014; Wong et al., 2013; Al-Mutairi 2011 and Kim et al., 2005) [13, 18, 4, 10]. *Proteus* species are the causative agents of a variety of opportunistic nosocomial infections including those of the respiratory tract, ear, nose, skin, burns and wounds. They are important pathogens of the urinary tract and primary infectious agents in patients with indwelling urinary catheters (Jacobsen et al., 2008 and Peerbooms et al., 1986) [9, 15]. *P. mirabilis* causes 90% of *Proteus* infections and is mostly found in people with compromised immune system (Mandal et al., 2015) [11]. Individuals suffering from urinary tract infections caused by *P. mirabilis* often develop cystitis, bacteruria, kidney and bladder stones, catheter obstruction due to stone encrustation and acute pylonephritis (Alatrash and Al-Yaseen, 2017) [2].

Poor water quality is responsible for the death of an estimated 5 million children in the developing countries. The detection of bacteria belonging to the genus *Proteus* in drinking water disqualifies its suitability for consumption due to its fecal pollution, which would threaten with waterborne infections (Drzewiecka, 2016) [8]. The pathogenesis of these bacteria is associated with possessing many virulence factors which include the pili (fimbriae), flagella, urease, protease, hemolysin etc. The urease and protease

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enzymes are produced by all strains of Proteus species and it is also considered as diagnostic and differential feature which characterizes the members of this genus from the rest of the intestinal family members (Ali and Yousif, 2015)\textsuperscript{[3]}
Therefore, the present study was planned to perform the isolation of \textit{P. mirabilis} from different sources and also to characterize their virulence gene profiles.

2. Materials and methods
2.1 Standard control and primers
ATCC (American Type Culture Collection) culture of \textit{Proteus mirabilis} (ATCC 12453) was used as standard control. Oligonucleotide primers were custom synthesized from M/s. Bioserve Biotechnologies Pvt. Ltd. (Hyderabad).

2.2 Sample collection
A total of 507 samples were collected from different sources of raw meats including chicken samples (98), mutton (45) and pork (72) from retail shops and slaughter houses, poultry cloacal swabs (151) from Livestock Farm Complex (LFC), NTRCVSc, Gannavaram and pig rectal swabs (37) from Dept. of Livestock Products Technology, NTRCVSc, Gannavaram, human urine samples (65) and human diarrhoeic samples (12) from clinical laboratories and water samples (27) from areas in and around Gannavaram, Krishna district, Andhra Pradesh. The samples collected were inoculated into 5ml TSB and incubated at 37 °C for 24 h for enrichment. Enriched samples were streaked on MacConkey agar, nutrient agar and blood agar plates and incubated at 37 °C for 24 h. Isolates were identified depending on morphological and biochemical tests and further confirmed by uniplex PCR.

2.3 DNA extraction from enriched broth samples
DNA was extracted by boiling and snap chilling method (Suresh et al., 2018)\textsuperscript{[17]}. A 1.5 ml of enriched broths were taken into micro centrifuge tubes and centrifuged at 8000 rpm for 10 min. Supernatant was discarded, 50 μl of nuclease free water was added and placed in boiling water bath at 100 °C for 10 min then immediately snap chilled for 10 min and centrifuged at 10,000 rpm for 5 min. The supernatant was taken as template and subjected to PCR and the PCR products were subjected to gel electrophoresis using 1.5% agarose with ethidium bromide as fluorescent dye and visualized using Gel Documentation unit (BIORAD, USA).

2.4 Molecular confirmation of \textit{Proteus mirabilis} by PCR
\textit{P. mirabilis} isolated from different samples by cultural methods were confirmed using \textit{P. mirabilis} specific PCR targeting \textit{ureR} gene (Table-1). PCR assay was optimized in 25 μl reaction mixture containing 2 μl of DNA template, 2.5 μl of 10x master mix (Go Taq Green Master Mix, Promega), 1.5 μl each of forward and reverse primers (10 pmol/μl) and the rest of the volume is made by adding nuclease free water. The cycling conditions were as follows: initial denaturation at 94°C for 4 min; 30 cycles of 94°C for 40 s, 58°C for 1 min and 72°C for 20 sec and a final elongation step at 72°C for 10 min (Zhang et al., 2013)\textsuperscript{[19]}.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'-3')</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{ureR}-F</td>
<td>GGTGAGATTGTATAATGG</td>
<td>225</td>
</tr>
<tr>
<td>\textit{ureR}-R</td>
<td>ATAAATCTGGAGAATGCGG</td>
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2.5 Detection of virulence genes in \textit{P. mirabilis} isolates
All the confirmed \textit{P. mirabilis} isolates from different sources were screened for the presence of virulence genes such as \textit{ureA}, \textit{ureC}, \textit{hpmA}, \textit{flaA} and \textit{zapA} (Table-2). PCR assay was optimized in 25 μl reaction mixture containing 3 μl of DNA template, 2.5μl of 10x master mix (Go Taq Green Master Mix, Promega), 0.35μl each of forward and reverse primers (10 pmol/μl) and the rest of the volume is made by adding nuclease free water, under standardized cycling conditions (Ali and Yousif, 2015)\textsuperscript{[3]}.

3. Results and Discussion
In the present study, a total of 507 samples from various sources viz. foods of animal origin (215), faecal samples (188), human clinical samples (77) and water samples (27) were analyzed for isolation and identification of \textit{P. mirabilis}. Out of 507 samples, 175 were positive for \textit{P. mirabilis} both by cultural and biochemical tests (Fig 1). The overall prevalence rate of \textit{P. mirabilis} was 34.51%. Out of 215 meat food samples analyzed, the highest rate of \textit{P. mirabilis} isolates was recovered from chicken samples (38.7%) followed by pork (37.5%) and mutton (28.9%). These prevalence rates were almost similar to the findings of Kim et al. (2005)\textsuperscript{[10]} who reported an overall prevalence of about 20-35% in raw meat samples.

\[ ~20\% ~\]
Regarding human clinical samples, out of 65 urine samples screened, 7 were positive for *P. mirabilis* showing prevalence rate of 10.7%, which was slightly less when compared with findings of Alatras and Al-Yaseen (2017) [5] who reported a prevalence rate of 19.3% in human urine samples and Ali and Yousif (2015) [3] who reported an overall prevalence of 17.6% (Fig 2). Less prevalence in our study can be attributed to the variation due to many factors such as geographic area, type of sampling, antibiotic usage etc.

The prevalence of *P. mirabilis* in chicken samples in the present study was 38.7%, which is in agreement with the findings of Kim et al. (2005) [10] who also reported 34% prevalence in chicken meat. Out of 38 *P. mirabilis* isolates recovered from chicken samples, all the five genes (*ureC, ureA, flaA, hpmA* and *zapA* genes) were found in 127 isolates (32.9%). Eight isolates (21.05%) showed *ureC*, *ureA*, *flaA* and *zapA* genes, eight isolates (21.05%) showed *ureA* and *ureC* genes, four isolates (10.52%) showed *ureC* and *flaA* genes.

The prevalence of *P. mirabilis* in chicken cloacal swabs was 49% in the present study, while 39% prevalence of *P. mirabilis* in poultry droppings was reported by Nahar et al. (2014) [13] in Bangladesh. The higher prevalence of *P. mirabilis* in chicken cloacal swabs might be due to unsanitary maintenance of broiler farms. Out of 74 *P. mirabilis* isolates recovered from poultry cloacal swabs, all the five genes (*ureC, ureA, flaA, hpmA* and *zapA* genes) were found in 22 isolates (29.62%). Three isolates (11.11%) showed *ureC* and *ureA* genes, five isolates (6.75%) showed *ureC* and *flaA* genes, three isolates (4.05%) showed *ureC* and *hpmA* genes, two isolates (2.7%) showed *ureC* and *zapA* genes, five isolates (6.75%) showed only *ureC* gene, four isolates (5.4%) showed only *ureA* gene, one isolate (1.35%) showed only *hpmA* gene.

The investigation on virulence properties of potentially pathogenic bacteria in animals and foods of animal origin was essential to consumer’s safety. For the detection of virulence pathogenic bacteria in animals and foods of animal origin was attempted during slaughter of pigs. Out of 27 *P. mirabilis* isolates recovered from pork samples, all the five genes (*ureC, ureA, flaA, hpmA* and *zapA* genes) were found in eight isolates (29.62%). Three isolates (11.11%) showed *ureC* and *ureA* genes, one isolate (3.7%) showed *ureA*, *flaA* and *zapA* genes, two isolates (7.4%) showed *ureC* and *zapA* genes and one isolate (3.7%) showed only *ureC* gene. Out of 14 *P. mirabilis* isolates recovered from pig rectal swabs, all the five genes
(ureC, ureA, flaA, hpmA and zapA genes) were found in four isolates (28.57%). Five isolates (35.71%) showed ureC, ureA and flaA genes, three isolates (21.42%) showed ureC and zapA genes, one isolate (7.14%) showed ureA and zapA genes and one isolate (7.14%) showed only ureA gene.

The prevalence of *P. mirabilis* in mutton was 28.9%, which is in perfect correlation with the findings of Dabassa (2013) [7] and Begum (1984) [8] who reported a prevalence of 29.7% and 26.6% in mutton in Jimma town, Southwest Ethiopia and Islamabad, respectively. Out of 13 *P. mirabilis* isolates recovered from mutton samples, all the five genes (ureC, ureA, flaA, hpmA and zapA genes) were found in three isolates (23.07%). One isolate (7.69%) showed ureC, flaA and zapA genes, six isolates (46.15%) showed ureC and ureA genes and three isolates (23.07%) showed only flaA gene.

The prevalence of *P. mirabilis* in human urine samples in the present study was 10.7% and all human diarrhoeal samples were negative for *P. mirabilis*. The results were in agreement with the findings of Philips (2014) [19] who reported a prevalence of 14.59% of *P. mirabilis*. In contrast, Nachammay et al. (2015) [12] reported a lower prevalence of *P. mirabilis* (4.8%) in urine samples of humans. Out of 7 *P. mirabilis* isolates recovered from human urine samples, none of them showed all the five genes in a single isolate. Four isolates (57.14%) showed ureC, ureA and flaA genes, two isolates (28.57%) showed ureA, ureC, zapA and hpmA genes and one isolate (14.28%) showed only flaA gene.

The prevalence of *P. mirabilis* in water samples in the present study was 7.4%. These results were less when compared with findings of Adesoji and Ogunjobi (2016) [1] who reported an overall prevalence of 18.18% in selected drinking water distribution channels in Southwestern Nigeria. Less prevalence in the present study can be attributed to the variation due to many factors such as geographic area, type of sampling and disinfection of water.

Less prevalence rates of the target virulence genes was observed in the present study compared to the findings of Ali and Yousif (2015) [10] who isolated *P. mirabilis* from human clinical samples in Iraq and reported higher prevalence rates of the ureC (100%), ureA (96.66%), flaA (86.66%), hpmA (100%) and zapA (100%) genes and the prevalence rates of ureC and zapA genes in our study were in agreement with the findings of Pathirana et al. (2018) [14] who isolated *Proteus* spp. from humans and pet turtles and reported 52% and 91.7% of prevalence for zapA and ureC genes, respectively. Due to paucity of reports on virulence gene profiles of *P. mirabilis* isolated from animals and animal foods, the results could not be compared.

4. Conclusion
This study shows the presence of *P. mirabilis* and its distribution in livestock, foods of animal origin, humans and water samples in India. The presence of *P. mirabilis* in animal faeces could act as a potential source of contamination of carcass during slaughter and may pose threat to food safety and human health, as it has been associated with enteritis in humans. The results obtained in this study highlighted the virulence gene profiles of *P. mirabilis* of animal and human origin.

5. Acknowledgment
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6. References
16. Philips O. Antibiogram study of *Proteus* spp. bacterial isolates from uropathogenic infections in University of Benin Teaching Hospital, Nigeria. Current Research in